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Microbial Evaluation of Coliphages in Various Water Systems in Riyadh, Saudi Arabia

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Coliphages viruses were monitored in conjunction with fecal coliform bacteriacoliphages, and the possible fecal contamination sources of Al-Hair Canal in various water systems from Southwestern Riyadh, Saudi Arabia, during the period between November 9th and March 2nd 2012 were identified. Coliphages were detected by the direct plate assay and using *Escherichia coli* as host bacteria. The membrane filter method was used for the fecal coliform detection on mFC culture media. Fecal coliforms were present on 100% of the samples against 65% on coliphages and a ratio of 62:1 fecal coliform to coliphage was calculated on 100 mL samples (n=72). As coliphages were proposed as possible indicators of enteric viruses, our study suggests that their use as indicators of faecal pollution with traditional coliform indicators and the implementation of treatment measures more effective in virus removal in re-used wastewater. Examination by electronic microscope of the observed selected phage lysates were for phages with icosahedral head, collar and contracted tail in ¢1, ¢2, ¢4, ¢5, ¢6 isolated phages thus belonging to family Myoviridae. Isolated Phage ¢3 was with icosahedral head and short tail characteristic of family Podoviridae. These results lead to the conclusion that the coliphage indicator could be an effective tool to evaluate and detect fecal contamination in various water systems.

Key word: Coliphages, Fecal contamination, Al-Hair Canal, Saudi Arabia.

Water is a natural resource that is universally needed for our daily activities. Water has been constantly exposed to various pollutants by bacteria and other forms of microorganisms. These microorganisms are ubiquitous in nature that they are widely distributed in nature both in the aquatic and terrestrial environment including the air[11]. Water is diversified in nature as it may be natural waters which can be grouped into: (a) Atmospheric waters such as rain, hail and snow;) b) Surface waters such as stream, ponds, lakes, rivers and estuaries and oceans; (c) Ground (or underground) such as waters spring, well, underground streams. While artificial waters, all of which are surface waters include: reservoirs, dams, oxidation ponds and man-made lakes[1,12].

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Coliphages were proposed to be indicators of water pollution and as possible models for enteroviruses during treatment of drinking water and wastewater[15]. Many earlier studies suggested that coliphages and enteroviruses could be removed at comparable rates during treatment processes, that certain coliphages are at least as resistant to environmental stresses and to chlorination as enteroviruses, and that coliphages exhibit a seasonal variation similar to that of enteroviruses[13]. Also, both coliphages and enteroviruses were found in chlorinated drinking water, sometimes in the same samples[1]. However, others studies reported that coliphage concentrations may increase under suitable environmental conditions, that coliphages may be present in the absence of detectable viruses, and, vice versa, that enteroviruses may be present in the absence of coliphages[10]. Coliphage concentrations were determined at several points

during drinking-water treatment at a plant located in southern Michigan. This included the water source and treated water after flocculation-sedimentation, sand filtration, and chlorination. The conditions of the treatment were previously described[5]. Coliphage concentrations in the water source were determined both by direct plaquing of grab samples and by plaquing samples concentrated by a filter adsorption-elution technique used to concentrate enteroviruses[4]. Treated water samples were examined for coliphages only after concentration.

Microorganisms generally may be beneficial or nonbeneficial. Beneficial are useful for various clinical, pharmaceutical and industrial processes apart from those that participate naturally as normal flora for proper functioning of human body system. On the contrary those that act as pathogen present in our environment especially as water contaminants could pose deleterious threat to human health and causation of epidemics[1,6,7]. Various activities of man both at the domestic and industrial level leads to contamination of water which has served as viable habitat for many types of microorganisms[3]. Contamination of drinking water with feacal and other materials may increase the risk of disease transmission to consumers[9]. Similarly, group of organisms such as coliforms may serve as indicator organisms to monitor our water resources and assess their quality[8].

MATERIALS AND METHODS

Sampling

Water samples were collected from Al-Hair Canal, Southwestern Riyadh, Saudi Arabia; 2424° North , 4650° East of the city of Riyadh and away from about 17 km. These samples were tested for fecal coliforms and coliphages during the period between November 9th and March 2nd 2012. The fecal coliforms and coliphage densities were evaluated and statistically compared to the determined possible relationships between indicators and to the determined possible sources of contamination through various water systems. Water sources were collected from within the rural areas of the Riyadh region, such as open wells, tube wells, gravity feed water supply systems and small scale estate water supply systems, canals of irrigation system and treated wastewater[19] . All samples were carried out taking the standard precautions to avoid contamination during sampling. Preparation of samples for analyses for coliphages were normally carried out in the field and kept at 4°C for <8 h until the indicator micro-organisms were quantified as described above. As a quality control procedure the temperature and pH were measured on different sampling points to evaluate their change parameters through the various water systems and verify if those changes have affected the fecal coliforms and coliphage densities measured on this project. The fecal coliforms and coliphage densities were evaluated and statistically compared to the determined possible relationships between indicators and the determined possible sources of contamination through water systems.

Bacterial enumeration

The phage sensitivity of 4 types of *Eschrichia coli* strains to the lytic section of coliphages was tested using the double-agar-layer (DAL). The strains were purchased by (Felix d'herrelle Reference center for Bacterial viruses, Quebec, Canada). The selected strains were *E.coli 11303, E.coli 0157, H7 E8229-83, C-7685-84, C-91-84, CL40* and *E. coli* 8 locally isolated strain from Riyadh, Saudi Arabia.

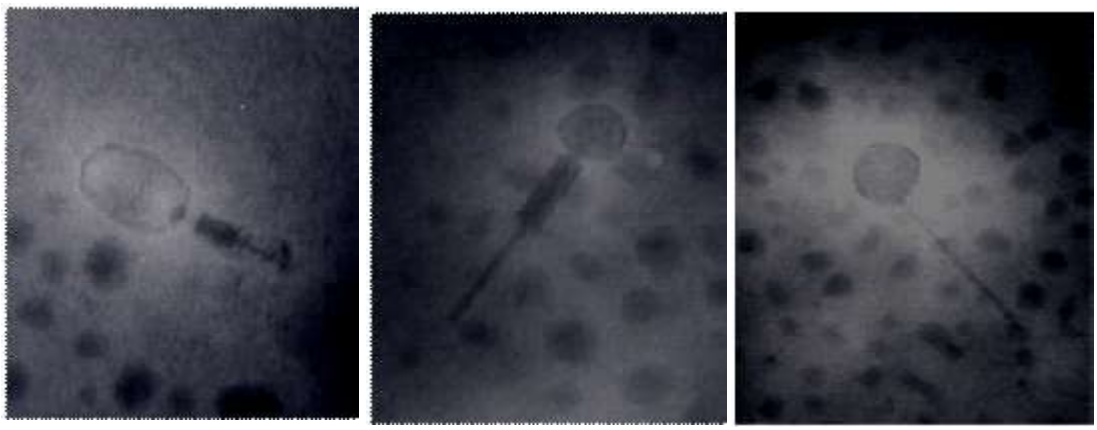
Bacteriophage enumeration

Phage detection in environmental samples consists of concentrating the sample using one of several published procedures, decontaminating the concentrate, and carrying out the phage assay by the double or single-layer methods. A wide range of bacterial host cells have been used as some are more efficient than others in hosting phages. Somatic coliphages can be assayed on an *E. coli C host*. The U.S. EPA has proposed two methods (methods # 1601 and 1602) to detect somatic coliphages *host is E. coli CN-13* and F specific coliphages *host is E. coli F-amp* in aquatic environments. Method 1601 include an overnight enrichment step water is supplemented with the host, MgCl2 and tryptic soy broth followed by "spotting" onto a host bacterial lawn. In Method 1602, a 100-ml water sample is supplemented with MgCl2, host bacteria, and double-strength molten agar. The mixture is poured onto petri dishes and the plaques are counted after overnight incubation[21,22]. Plaques of different sizes

and morphology were selected for purification. The overnight culture of the host bacterium (1-2 ml) was sub cultured into 50ml of fresh liquid medium (N. Broth) and incubated with shaking at 37°C for about 1.5h. A will isolated plaque was then picked with a sterile Pasteur pipette and transfer into the host culture which was further incubated for 5-8hr. Bacterial cells were separated from the culture by centrifugation (4000rpm, 20min) and by membrane filtration with 0.45¼m pore size Millipore filters. The titer and purity of the phage lysate was determined by Ueda and Horan method[21] and the purification procedure was repeated 2-3 times to obtain a pure one-phage lysate. The morphology of phages was investigated using transmission electron microscope. Phage lysates were centrifuged at 40.000 rpm for 120 min for 4°C. Sediments were deposited on carbon-coated copper grids and stainal with 2% PTA (pH 7.2) and studied in a Joel 100-CX electron microscope.

RESULTS AND DISCUSSION

The above study was conducted to evaluate coliphages as indicators of fecal pollution, elucidate the occurrence of fecal coliforms and coliphages, and identify possible fecal contamination sources triplicate of artificial waters in various water systems from Riyadh, Saudi Arabia. As a result, the canal probably does not have any stationary source of fecal contamination and the virus and bacteria indicators detected may be coming from the fecal contamination of wastewater. The samples were tested for fecal coliforms and coliphages during the period between November 9th and March 2nd 2012. As a quality control procedure the temperature, pH were measured on different sampling points to evaluate their change parameters through the canal and verify if those changes have affected the fecal coliforms and coliphage densities measured on this project. The fecal coliforms and coliphage densities are evaluated and statistically compared to determine possible relationships between indicators and to determine possible sources of contamination through the canal. The densities of fecal coliforms and coliphages were expressed as 100 ml sample volumes. The fecal coliforms were present in 72 (100 %) of the samples against the coliphages that were present only in 47 (65 %) of the samples. A ratio of 62 fecal coliforms per coliphage (62:1) was calculated. The average of all densities of fecal coliforms and coliphages were statistically compared, with (n – 1) degrees of freedom and two tails alpha value of 0.05 (± = 0.05). Fecal coliforms resulted in a significant higher average value than coliphage densities. The occurrence of coliphage and fecal coliforms in this water was unprecedented since no previous research had been performed. The concentration of fecal coliforms was significantly higher (t70, 0.05 = 1.993. $p < 0.001$) than that of coliphages. The fecal coliforms were present in 100 % of the samples against the coliphages that were present only on 65 % of the samples and a ratio of 62 fecal coliforms per coliphage (62:1) was calculated. Measures of p^H and temperature were correlated with the indicators, coliphages and fecal coliforms. The correlation coefficient was used to measure the strength of the relationship between each indicator of water quality. The two fecal related indicators measured were significantly (t70, 0.05 = 1.993, $p <$ 0.001) positively correlated with each other, that is, when one increased in number, the other also increased in number. Changes in coliphage concentrations are apparently related to changes of fecal coliforms concentrations. Mean fecal coliform densities on each point were statistically compared against each other for coliphage and fecal coliform densities. The analysis concludes that fecal coliforms and coliphage average on different points are not significatively different (t11, 0.05 = 2.20). As a result, the canal probably does not have any stationary sources of fecal contamination, and maybe those indicators, virus and bacteria, that were detected are coming from the fecal contamination of the Al-Hair canal. Although statistical analysis did not determine possible areas of fecal pollution, the irrigation system may be polluted by animals such as dogs and goats. The presence of those warm blooded animals in the areas surrounding the canal of the irrigation system was very evident during sampling periods. However, scientifically, the true source of those indicators is still unknown. Coliphage densities correlate with the densities of the fecal indicator bacteria. This relationship between fecal coliforms and coliphage deserves future discussion because of the current status of the fecal coliform bacteria as an indicator. The results



(A)

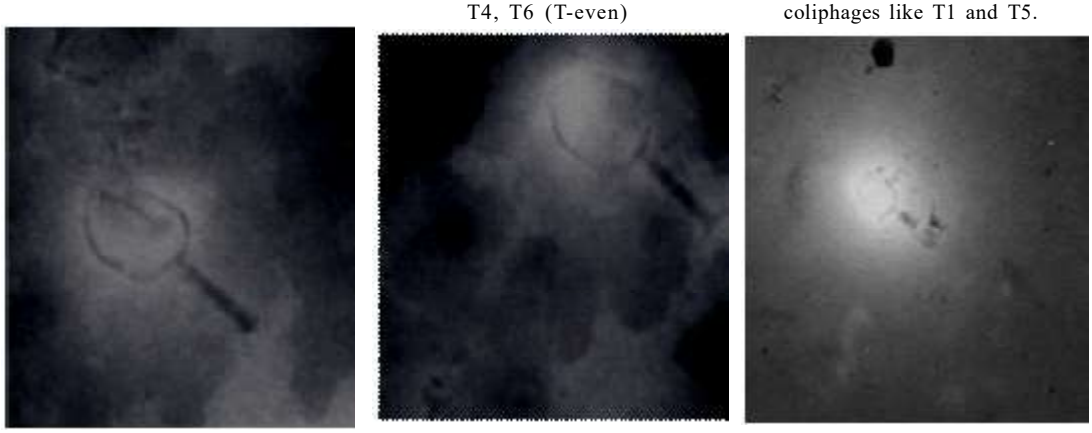
ds DNA, e.g. coliphages like T2, T4, T6 (T-even), Hexagonal head, rigid tail with a contractile sheath and tail fibers

(B)

Hexagonal head, rigid tail with a contractile sheath and tail fibers., ds DNA, coliphages like T2, T4, T6 (T-even)

(C)

Hexagonal head, flexible tail contractile sheath absent. Tail fibers present or absent, ds DNA, e.g. - coliphages like T1 and T5.



(D)

ds DNA, e.g. coliphages like T2, T4, T6 (T-even), Hexagonal head, rigid tail with a contractile sheath and tail fibers

(E)

ds DNA, e.g. coliphages like T2, T4, T6 (T-even), Hexagonal head, rigid tail with a contractile sheath and tail fibers

(F)

Hexagonal head, Tail shorter than head, contractile sheath absent. Tail fibers present or absent, ds DNA., e.g. coliphages T3 and T7

(G)

Only head made up of small cashmeres no tail. Ss DNA , e.g. coliphages f2, MS2.

A: ϕ^1, B: ϕ^2, C: ϕ^4, D: ϕ^5, E: ϕ^6, F: ϕ^6, G: ϕ^3

Fig. 1. Transmission electron micrographs of coliphages isolated from Al-Hair Canal

of this analysis suggest that a relationship or correlation exists. Results observed showed a significant relationship of coliphages and fecal coliforms densities, but this study failed to prove that coliphages were good indicators of fecal contamination because of a lack of comparative values in other waters.

Screening of water samples on sensitive *Eschrechia coli* lawns allowed the selection of six phage isolates with different plaque size and shape which were further propagated. Upon incubation of all these phage isolate with RNase (¼g/plate), all phages selected produced plaques in the presence of enzyme, indicating that they are DNA phages. Electronic microscope of phage lysates from water samples revealed several morphological patterns. All lysates examined were for tailed phages (Fig-1), however they could be further divided into different morph types by head shape and tail structure. The types observed were for phages with icosahedral head, collar and contracted tail in isolated phages ϕ^1, ϕ^2, ϕ^4, ϕ^5, ϕ^6 (Fig. 1, A, B, C, D, E and F), thus belonging to family Myoviridae. Isolated phage ϕ^3 with icosahedral head and short tail (Fig. 1, G) characteristic of family Podoviridae.

Our study suggests the use of coliphges an indicators of faecal pollution in addition to the traditionally used indicators and the implementation of treatment measures' more effective in virus removal in re-used wastewater. Coliphages could initially be differentiated based an plaque size and shape through such characters are not very reliable to classification purposes[9]. Electron microscopy is essential for studying phage ecology and for phage identification. our study revealed the abundance of tailed somatic phages of families Myoviridae, Poloviridae in agreement with the studies of and phage identification by electron microscopy is rapid and simple[8]. Identification by host range and plaque size is taxonomically unacceptable, and identification by antisera[15] is prohibitive, at least in tailed phages. Electron microscopy is of limited value in phages of uncharacteristic morphology, for example, actinophages[9]. In enterobacterial phages, of which are morphologically varied, it appears as the method of choice for phage identification. The above study was conducted to evaluate coliphages as indicators of fecal pollution, elucidate the occurrence of fecal coliforms and coliphages, and identify possible fecal contamination sources in the irrigation system of Al-Hair canal. Although the bacterial host for the phage isolated in this study was found to be similar to Enterohaemorrhgic *E.coli*, a common enteric bacteria belonging to the family Enterobacteriaceae. Is commonly found in sewage and has been associated with nosocomial infections in the gastrointestinal, urinary, respiratory, and biliary tracts of debilitated hospital patients. This organism represents an increased health risk because an important aspect of this organism's physiology is its ability to resist the affects of antibiotics typically prescribed to treat the infections it causes. Resistance is due to an inducible chromosomally encoded cephalosporinase that can inactivate cephamycins and cephalosporins[12].

The phage isolated in the author study has not been identified, a common phage that is known to infect Citrobacter sp. is the temperate phage Mu[9]. Unlike many other viruses, this phage has a broad host range that includes *E. coli*, *Salmonella*, and *Erwinia* plant pathogens. The potential for this phage to infect and kill several potentially harmful enteric bacteria without the application of other phage further supports its potential as a control agent. However, in order for this phage to be used as a control agent in Lake Pontchartrain it would have to be able to infect its host under brine water conditions. Another study suggest that the infection of the host by this phage is inhibited under brine water conditions[20]. The presence of coliform bacteria in aquatic environments indicates that the environment has been contaminated with fecal material originating from humans or other animals, billions of gallons of polluted water containing potentially dangerous levels of fecal coliforms[5]. Typically, when high numbers of coliforms are found in recreational or potable water sources, usage must be restricted to prevent a human or animal health crisis. Some bacteriophages were found to infect and multiply in species of closely related genera[11]. These polyvalent phages were suspected to cause possible biological control of overwhelming populations of the faecal coliforms in various water system. Studies on the incidence of phages in water environments have been reported from most parts

of the world for some decades now. Unfortunately the data are not particularly consistent and comparisons are generally not meaningful. One reason for this is that there are many variables that affect the incidence, survival and behavior of phages in different water environments, including the densities of both host bacteria and phages, temperature, p^H and so on[19]. The value of phages as models/surrogates for viruses has been applied in the routine monitoring of raw and treated drinking water supplies[8], and in the assessment of the efficiency of domestic point-of-use water treatment units. While they are useful and meet many of the basic requirements as surrogates for enteric viruses. This is underlined by the detection of enteric viruses in treated drinking water supplies which yielded negative results in tests for phages, even in presence-absence tests on 500 ml samples of water[8]. Many members of the total coliform group and some so-called faecal coliforms (e.g. species of *Klebsiella* and *Enterobacter*) are not specific to faeces, and even *E. coli* has been shown to grow in some natural aquatic environments[18] .Hence, the primary targets representing faecal contamination in temperate waters are now considered to be *E. coli* and enterococci. For tropical waters/soils, where *E. coli* and enterococci may grow, alternative indicators such as Clostridium perfringens may be preferable.

Based on these data and studies reported by these results lead to the conclusion that the coliphage indicator could be an effective tool to evaluate fecally contaminated water, since water could have fecal coliforms as natural environmental flora but the coliphages should come from a fecal source, so that the results of this study suggest that a valid assessment of the risk associated with contaminated recreational fresh water cannot be made using only coliphages as an indicator and the use of coliphage analysis can underestimate the number of fecal coliforms and the possible presence of environmental pathogens even in the absence of fecal pollution and it could also be underestimating the potential public health significance of pathogenic enteroviruses in water and fail to determine true fecally polluted water. It seems imperative to include coliphage assay as a water quality monitoring method to ensure that no microbiological population can affect human health. Perhaps multiple methodologies can be used in combination to ensure the safety of water, virological and bacteriological. This strategy could help to avoid searching for an indicator that meets all criteria.

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